

What I claim is:

1. A purified nucleic acid segment comprising a coding region encoding enzymatically active chondroitin synthase.
2. The purified nucleic acid segment of claim 1, wherein the purified nucleic acid segment encodes a chondroitin synthase isolated from *Pasteurella multocida*.
3. The purified nucleic acid segment of claim 2, wherein the purified nucleic acid segment encodes the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.
4. The purified nucleic acid segment of claim 2, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1 or 3.
5. A purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase, wherein the purified nucleic acid segment is capable of hybridizing to the nucleotide sequence of SEQ ID NO:1 or 3.

6. A purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase, wherein the purified nucleic acid segment has semiconservative or conservative amino acid changes or is a truncated segment when compared to the nucleotide sequence of SEQ ID NO:1 or 3.

7. A recombinant vector selected from the group consisting of a plasmid, cosmid, phage, integrated cassette or virus vector and wherein the recombinant vector further comprises a purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase.

8. The recombinant vector of claim 7, wherein the purified nucleic acid segment encodes the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

9. The recombinant vector of claim 7, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

10. The recombinant vector of claim 7, wherein the plasmid further comprises an expression vector.

11. The recombinant vector of claim 10, wherein the expression vector comprises a promoter operatively linked to the enzymatically active *Pasteurella multocida* chondroitin synthase coding region.

12. A recombinant host cell, wherein the recombinant host cell is a prokaryotic cell transformed with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase.

13. The recombinant host cell of claim 12, wherein the purified nucleic acid segment encodes the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

14. The recombinant host cell of claim 12, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

15. The recombinant host cell of claims 13 or 14, wherein the host cell produces chondroitin.

16. The recombinant host cell of claim 12, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified structure.

17. The recombinant host cell of claim 12, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified size distribution.

18. A recombinant host cell, wherein the recombinant host cell is an eukaryotic cell transfected with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase.

19. The recombinant host cell of claim 18, wherein the purified nucleic acid segment encodes the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

20. The recombinant host cell of claim 18, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

21. The recombinant host cell of claims 19 or 20, wherein the host cell produces chondroitin.

22. The recombinant host cell of claim 18, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified structure.

23. The recombinant host cell of claim 18, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified size distribution.

24. A recombinant host cell, wherein the recombinant host cell is electroporated or transformed to introduce a recombinant vector into the recombinant host cell, wherein the recombinant vector comprises a purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase.

25. The recombinant host cell of claim 24, wherein the purified nucleic acid segment encodes the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

26. The recombinant host cell of claim 24, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

27. The recombinant host cell of claim 25 or 26, wherein the host cell produces chondroitin.

28. The recombinant host cell of claim 24, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified structure.

29. The recombinant host cell of claim 24, wherein the enzymatically active *Pasteurella multocida* chondroitin synthase is capable of producing a chondroitin polymer having a modified size distribution.

30. A recombinant host cell, wherein the recombinant host cell is transduced with a recombinant vector comprising a purified nucleic acid segment having a coding region encoding enzymatically active *Pasteurella multocida* chondroitin synthase.

31. The recombinant host cell of claim 30, wherein the purified nucleic acid segment encodes the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

32. The recombinant host cell of claim 30, wherein the purified nucleic acid segment comprises a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

33. The recombinant host cell of claim 31 or 32, wherein the host cell produces chondroitin.

34. The recombinant host cell of claim 30, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified structure.

35. The recombinant host cell of claim 30, wherein the enzymatically active chondroitin synthase is capable of producing a chondroitin polymer having a modified size distribution.

36. A purified composition, wherein the purified composition comprises an enzymatically active chondroitin synthase polypeptide.

37. A purified composition, wherein the purified composition comprises a chondroitin polymer made by a recombinant process.

38. A purified composition, wherein the purified composition comprises a chondroitin polymer made by a chondroitin synthase.

39. A purified composition, wherein the purified composition comprises a chondroitin polymer made by a *Pasteurella multocida* chondroitin synthase.

40. A purified composition, wherein the purified composition comprises a chondroitin polymer made by the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

41. A purified composition, wherein the purified composition comprises a chondroitin polymer made by the *Pasteurella multocida* chondroitin synthase comprising the nucleotide sequence in accordance with SEQ ID NO:1 or 3.

42. The purified composition of claims 36, 37, 38, 39, 40, 41, and 42, wherein the chondroitin polymer is represented by a structure, (Beta-1,4-GlcUA-beta-1,3-GalNAc)<sub>n</sub> wherein n is a positive integer greater than or equal to 1.



43. A purified composition, wherein the purified composition comprises a chondroitin polymer having a modified size distribution.

44. A purified composition, wherein the purified composition comprises a chondroitin polymer having a modified structure.

45. The purified composition of claim 42, wherein the chondroitin polymer is unsulfated.

46. A method for producing a chondroitin polymer *in vitro* comprising the steps of:

- providing a chondroitin synthase;
- placing the chondroitin synthase in a medium suitable for the expression of a chondroitin polymer; and
- extracting the chondroitin polymer out of the medium.

47. The method of claim 46, wherein in the step of providing a chondroitin synthase, the chondroitin synthase is from *Pasteurella multocida*.

48. The method of claim 47, wherein in the step of providing a chondroitin synthase, the chondroitin synthase is from *Pasteurella multocida* and has an amino acid sequence in accordance with SEQ ID NO:2 or 4.

49. The method of claim 47, wherein in the step of providing a chondroitin synthase, the chondroitin synthase from *Pasteurella multocida* is a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

50. A method for producing a chondroitin polymer *in vivo*, comprising the steps of:

- providing a chondroitin synthase;
- placing the chondroitin synthase in a native or recombinant organism, thereby providing a native or recombinant organism having a chondroitin synthase there;
- placing the native or recombinant organism having a chondroitin synthase therein in a medium suitable for the expression of a chondroitin polymer; and
- extracting the chondroitin polymer.

51. The method of claim 50, wherein in the step of providing a chondroitin synthase, the chondroitin synthase is from *Pasteurella multocida*.

52. The method of claim 51, wherein in the step of providing a chondroitin synthase, the chondroitin synthase is from *Pasteurella multocida* and has an amino acid sequence in accordance with SEQ ID NO:2 or 4.

53. The method of claim 51, wherein in the step of providing a chondroitin synthase, the chondroitin synthase from *Pasteurella multocida* is a nucleotide sequence in accordance with SEQ ID NO:1 or 3.

54. The purified composition of claim 42, wherein the chondroitin polymer is sulfated *in vitro*.

55. The purified composition of claim 42, wherein the chondroitin polymer is epimerized *in vitro*.

56. A recombinant host cell containing a chondroitin synthase and an epimerase.

57. A recombinant host cell containing a chondroitin synthase and a sulfotransferase.

58. A recombinant host cell containing a chondroitin synthase, an epimerase, and a sulfotransferase.

59. The purified composition of claim 42, wherein the chondroitin polymer is sulfated *in vivo*.

60. The purified composition of claim 42, wherein the chondroitin polymer is epimerized *in vivo*.

61. The recombinant host cell of claims 56, 57, or 58, wherein the chondroitin synthase is a *P. multocida* chondroitin synthase.

62. The recombinant host cell of claim 61, wherein the *P. multocida* chondroitin synthase is in accordance with SEQ. ID NO:2.

63. A purified composition, wherein the purified composition comprises an enzymatically active chondroitin synthase polypeptide.

64. A method for detecting a DNA species, comprising the steps of:

- obtaining a DNA sample;

- contacting the DNA sample with a purified nucleic acid segment in accordance with SEQ ID NO:1 or 3;
- hybridizing the DNA sample and the purified nucleic acid segment thereby forming a hybridized complex; and
- detecting the complex.

65. A method for detecting a bacterial cell that expresses mRNA encoding *Pasteurella multocida* chondroitin synthase, comprising the steps of:

- obtaining a bacterial cell sample;
- contacting at least one nucleic acid from the bacterial cell sample with a purified nucleic acid segment in accordance with SEQ ID NO:1 or 3;
- hybridizing the at least one nucleic and the purified nucleic acid segment thereby forming a hybridized complex; and
- detecting the hybridized complex; wherein the presence of the hybridized complex is indicative of a bacterial strain that expresses mRNA encoding *Pasteurella multocida* chondroitin synthase.

66. A method for producing a chondroitin polymer, comprising the steps of:

- introducing a purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase into a host organism, wherein the host organism contains nucleic acid segments encoding enzymes which produce UDP-GlcUA and UDP-GalNAc;
- growing the host organism in a medium to secrete chondroitin polymer; and
- recovering the secreted chondroitin polymer.

67. The method according to claim 66, wherein in the step of recovering the chondroitin polymer, the chondroitin polymer is extracted from the medium or the cells or combinations thereof.

68. The method according to claim 67, further comprising the steps of  
purifying the extracted chondroitin polymer.

69. The method according to claim 66, further comprising the step of sulfating the chondroitin polymer.

70. The method according to claim 66, further comprising the step of epimerizing the chondroitin polymer.

71. The method according to claim 66, wherein in the step of growing the host organism, the host organism secretes a structurally modified chondroitin polymer.

72. The method according to claim 66, wherein in the step of growing the host organism, the host organism secretes a chondroitin polymer having a modified size.

73. A pharmaceutical composition comprising a preselected pharmaceutical drug and an effective amount of a chondroitin polymer produced by a chondroitin synthase directly or after modification of the polymer by sulfation or epimerization or combinations thereof.

74. The pharmaceutical composition of claim 73, wherein the chondroitin synthase is from *Pasteurella multocida*.

75. The pharmaceutical composition of claim 74, wherein the chondroitin polymer is produced by the *Pasteurella multocida* chondroitin synthase of SEQ ID NO:2 or 4.

76. The pharmaceutical composition of claim 73, wherein the molecular weight of the chondroitin polymer is modified thereby producing a modified molecular weight pharmaceutical composition.

77. The pharmaceutical composition of claim 73, wherein the molecular weight of the chondroitin polymer is modified thereby producing a modified molecular weight pharmaceutical composition capable of targeting a specific tissue or cell type within a patient having an affinity for the modified molecular weight pharmaceutical composition.

78. A purified and isolated nucleic acid sequence encoding enzymatically active chondroitin synthase, the nucleic acid sequence selected from the group consisting of:

- the nucleic acid sequence in accordance with SEQ ID NO:1 or 3;
- complementary nucleic acid sequences to the nucleic acid sequence in accordance with SEQ ID NO:1 or 3;
- nucleic acid sequences which will hybridize to the nucleic acid sequence in accordance with SEQ ID NO:1 or 3; and
- nucleic acid sequences which will hybridize to the complementary nucleic acid sequences of SEQ ID NO:1 or 3.



79. A purified and isolated nucleic acid segment consisting essentially of a nucleic acid segment encoding enzymatically active chondroitin synthase.

80. A procaryotic or eucaryotic host cell transformed or transfected with an isolated nucleic acid segment according to claim 1, 2, or 3 in a manner allowing the host cell to express chondroitin polymer.

81. An isolated nucleic acid segment consisting essentially of a nucleic acid segment encoding chondroitin synthase having at least one nucleic acid segment sufficiently duplicative of the nucleic acid segment in accordance with SEQ ID NO:1 or 3 to allow possession of the biological property of encoding for a *Pasteurella multocida* chondroitin synthase.

82. A cDNA sequence according to claim 81.

83. A prokaryotic or eukaryotic host cell transformed or transfected with a nucleic acid segment according to claim 81 in a manner allowing the host cell to express chondroitin polymer.

84. A purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase, wherein the purified nucleic acid

segment is capable of hybridizing to the nucleotide sequence in accordance with SEQ ID NO:1 or 3.

85. A recombinant host cell that is the product of a process comprising the steps of:

- i. providing a culture comprised of host cells comprising a chondroitin synthase promoter which is endogenous to the host cells and a chondroitin synthase gene under transcriptional control of the chondroitin synthase promoter;
- ii. transforming the host cells in the culture with a heterologous DNA molecule, comprising:
  - (1) two genetic elements assembled such that the coding regions of both elements are translated in the same direction, wherein the downstream genetic element comprises a selectable marker gene, a promoter that controls the transcription of the selectable marker gene, and a transcription termination sequence, and wherein the upstream genetic element comprises one or more promoterless coding regions encoding at least one desired polypeptide followed by a transcription termination sequence,

- (2) sequences that flank the genetic elements and are oriented such that their direction of translation is the same as that of the two heterologous genetic elements, and
- (3) sequences that flank the genetic elements and are sufficiently homologous to the chondroitin synthase gene to enable integration by homologous recombination,

whereby integration of the genetic elements into the chondroitin synthase gene results by means of homologous recombination;

- iii. selecting for host cells produced in step (b) that express said selectable marker polypeptide; and
- iv. screening and extracting the host cells obtained in step (c) to obtain host cells that produce chondroitin synthase.

86. The recombinant host cell of claim 85, wherein the recombinant host cell is a vector.

87. The recombinant host cell of claim 85, wherein the chondroitin synthase gene is obtained from *Pasteurella multocida*.

88. The recombinant host cell of claim 87, wherein the chondroitin synthase gene obtained from *Pasteurella multocida* is in accordance with SEQ ID NO:1 or 3.

89. The recombinant host cell of claim 85, wherein the recombinant host cell expresses chondroitin synthase.

90. The recombinant host cell of claim 89, wherein the recombinant host cell expresses a chondroitin synthase in accordance with SEQ ID NO:2 or 4.

91. The recombinant host cell of claim 85, further comprising the steps of: (e) exposing the host cells identified in step (d) to a mutagen under conditions such that mutations are created in the chondroitin synthase gene; and (f) testing the host cells produced in step (d) or (e) for host cells that produce the marker polypeptide at a higher level than the initial level, to obtain a recombinant host cell having a mutation that causes increased expression of the upstream genetic element resulting in an increase in production by the host cells of all polypeptides encoded by said heterologous DNA molecule compared to the production of all polypeptides encoded by the heterologous DNA molecule by the host cells in the absence of the mutation, wherein the increased

expression is retained in the absence of conditions that select for cells having the increased expression.

92. A recombinant method for producing a heterologous polypeptide in a host cell, comprising the steps of:

- (a) transforming the host cell with a vector comprising a promoter and a nucleic acid construct comprising a nucleic acid sequence encoding a desired heterologous polypeptide, wherein said promoter comprises a transcriptional activating region of the nucleic acid sequence set forth in SEQ ID NO:1 or 3, and wherein the nucleic acid construct is positioned in operable linkage with the promoter;
- (b) culturing the transformed host cell of step (a); and
- (c) recovering the heterologous polypeptide from the transformed host cell of step (b).

93. A process for producing a chondroitin polymer by fermentation of a cell expressing a chondroitin synthase enzyme having an amino acid sequence in accordance with SEQ ID NO:2 or 4.

94. A process for the *in vitro* sulfation of a chondroitin polymer, wherein the chondroitin polymer is produced by a chondroitin synthase, wherein the chondroitin polymer is sulfated by either chemical or enzymatic means.

95. The process of claim 94, wherein the chondroitin synthase is a *Pasteurella multocida* chondroitin synthase.

96. The process of claim 95, wherein the *Pasteurella multocida* chondroitin synthase is in accordance with SEQ ID NO:2 or 4.

97. A dermatan polymer obtained by the process of epimerizing a chondroitin polymer, wherein the chondroitin polymer is produced by a chondroitin synthase.

98. The dermatan polymer of claim 97, wherein the chondroitin synthase is a *Pasteurella multocida* chondroitin synthase.

99. The dermatan polymer of claim 98, wherein the *Pasteurella multocida* chondroitin synthase is in accordance with SEQ ID NO:2 or 4.

100. A recombinantly produced unsulfated chondroitin polysaccharide.

101. The recombinantly produced unsulfated chondroitin polysaccharide of claim 100 produced by a chondroitin synthase.

102. The recombinantly produced unsulfated chondroitin polysaccharide of claim 101, wherein the chondroitin synthase is in accordance with SEQ ID NO:2 or 4.

103. A polysaccharide comprising alternating Beta 1,4-linked GalNAc and Beta 1,3-linked GlcUA in a 1:1 ratio of the polysaccharide, the polysaccharide further having the properties: (1) nature: a white amorphous powder; (2) solubility: insoluble in alcohol, acetone, chloroform, and soluble in water and dimethylsulfoxide; (3) component sugars: glucuronic acid and N-acetyl-galactosamine only; (4) molecular weight: 1,000 - 250,000; (5) H-NMR spectrum: exhibiting signals characteristic of unsulfated chondroitin; (6) enzymatic sensitivity: susceptible to chondroitinase ABC, but not hyaluronate lyase; and (7) color reaction: positive to phenol-sulfuric acid reaction and carbazole reaction.

104. The polysaccharide of claim 103, wherein the polysaccharide is extracted and isolated from a culture media in which a microorganism belonging to the class *Pasteurella* is cultured.

105. The polysaccharide of claim 104, where the culture media is selected from the group consisting of tissue, yeast or milk extracts or a chemically defined media composed of vitamins, amino acids and salts, or combinations thereof.

106. The polysaccharide of claim 105, wherein the polysaccharide is purified from the culture media by a method selected from the group consisting of solvent precipitation, aliphatic quaternary amine precipitation, ion exchange chromatography, selective extraction, or selective ultrafiltration/dialysis, and combinations thereof.

107. A purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase, the purified nucleic acid segment corresponding to residues 45 to 704 of SEQ ID NOS:1 or 3.



108. A purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase, the purified nucleic acid segment corresponding to residues 75 to 704 of SEQ ID NOS: 1 or 3.

109. A purified nucleic acid segment having a coding region encoding enzymatically active chondroitin synthase, the purified nucleic acid segment corresponding to residues 1 to 704 of SEQ ID NOS: 1 or 3.

110. The polysaccharide of claim 106, wherein the polysaccharide is modified by a treatment selected from the group consisting of sulfation, epimerization, fragmentation, or cross-linking and combinations thereof.